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Award Number: W81XWH-12-1-0420

TITLE: An *in vivo* shRNA-drug screen to identify novel targeted therapy combinations for KRAS mutant cancers"

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REPORT DATE: September 2013

TYPE OF REPORT: Annual Report

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
September 2013	Annual	01-September-2012 to 31-August-2013

4. TITLE AND SUBTITLE
An in vivo shRNA-drug screen to identify novel targeted therapy combinations for KRAS mutant cancers

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-12-1-0420

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Massachusetts General Hospital
Boston, MA 02114-2554

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The primary shRNA-drug screen utilizing PDAC cell lines has been completed. The top 100 gene targets that specifically cooperate with MEK inhibitors were prioritized using the statistical RNAi gene enrichment ranking (RIGER) algorithm, which considers all shRNAs against a specific gene as a "hairpin set". Construction of the inducible shRNA "mini-pool" based on the top 100 gene hits from the primary screen is currently underway. Upon completion, this shRNA mini-pool will be utilized to perform the secondary shRNA-drug screen in an orthotopic mouse pancreatic cancer model as originally proposed.

15. SUBJECT TERMS
KRAS mutation, pancreatic cancer, MEK inhibitor

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	9	19b. TELEPHONE NUMBER *(include area code)*

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INTRODUCTION

Recent studies and results from early clinical trials with MEK and PI3K inhibitor combinations have supported the use of targeted therapy combinations for cancers harboring oncogenic KRAS mutations, which are found in ~90% of pancreatic ductal adenocarcinoma (PDAC) and ~20% of all human cancers. Large-scale functional genomic or "synthetic lethal" RNAi screens represent a potentially powerful tool for identifying novel gene targets for cancer therapy, but have two major weaknesses: (1) Most RNAi screens assess the effect of RNAi-mediated gene inhibition alone and have not been leveraged to identify potential combination therapies, a promising emerging clinical approach, and (2) RNAi screens are typically conducted *in vitro*, and do not address the effects of the *in vivo* tumor microenvironment and do not necessarily select for those targets most likely to produce the dramatic *in vivo* responses needed for clinical efficacy in patients. To address these deficiencies, we are pursuing a novel *in vivo* RNAi-drug screen approach utilizing mouse models of PDAC. We hypothesize that an RNAi screening and validation strategy geared to identify gene targets that produce the most dramatic responses *in vivo* when combined with a specific pharmacologic inhibitor will allow more rapid identification of novel targeted therapy combinations with the greatest likelihood of clinical efficacy.

BODY

Aim 1/Task 1:

The primary shRNA-drug screen utilizing PDAC cell lines has been completed, though some modifications were required to the initial plan. Technical limitations restricted our ability to perform the pooled shRNA-drug screen in xenografts as originally planned in the majority of PDAC cell lines, though eventually we were able to establish appropriate conditions to perform the shRNA screen in xenografts from two PDAC cell lines (MIAPACA2 and PA-TU-8988T). The major factor that limited our ability to perform the screen in xenografts was the efficiency with which most cell lines formed subcutaneous xenograft tumors. In order to achieve the desired coverage of at least 1000 cells per shRNA in the library, injection of 5-10 million cells into each mouse was required. However, for most cell lines, only a fraction of the cells injected survive to form a tumor. For cell lines with lower efficiencies of tumor formation, the surviving fraction of cells is low and insufficient to provide the necessary ratio of cells to shRNA required to generate quality shRNA screen data. Thus, only two cell lines with high tumor forming efficiency (MIAPACA and PA-TU-8988T) were eventually amenable to screening in xenografts.

Therefore, in order to move the project forward to the more important secondary screen using the inducible shRNA "mini-pool" in an orthotopic injection model, we performed our primary screen using an analogous in vitro approach in 6 total PDAC cell lines (including MIAPACA2 and PA-TU-8988T above) by previously described and proven methods (Corcoran et al, Cancer Cell 2013). This approach was considered the most reasonable alterative for several

reasons. First, as noted by one of our reviewers, the tumor microenvironment in a subcutaneous xenograft is not fully representative of the tumor microenvironment of a pancreatic tumor that forms de novo. Therefore, the need for the primary screen to be performed in vivo is of much less importance than in the secondary screen, in which tumors are injected orthotopically into the pancreas and develop more physiologically-relevant tumor microenvironments. Second, the in vitro shRNA-drug screen is a proven approach that we have used successfully to identify key genes that, when inhibited, cooperate with MEK inhibitors to kill KRAS mutant cancer cells. For example, this approach identified BCL-XL as a promising target for combination therapy with MEK inhibitors with robust preclinical activity (Corcoran et al, Cancer Cell 2013), and has led to the development of an NCI/CTEP-supported clinical trial of the BCL-XL inhibitor navitoclax and the MEK inhibitor trametinib in patients with KRAS mutant cancers with expansion cohorts in pancreatic, colorectal and lung cancer (NCT02079740; Ryan Corcoran, PI) that is currently enrolling patients. Since this approach has proven effective in the past, we reasoned that promising gene targets identified through this approach could then be further prioritized for relevance using the planned secondary screen in orthotopic tumors with physiologically-relevant tumor microenvironments. Finally, we believe that the majority of gene targets that would have profound in vivo cooperativity with MEK inhibitors would be likely to show at least some promising activity in the in vitro assay, as well. Thus, a reasonable alternative approach would be to identify a manageable number of promising targets through an in vitro approach and then to determine which of these targets exhibits the most striking in vivo activity with MEK inhibitors in the orthotopic secondary screen.

Gene hits from the primary screen were prioritized using the statistical RNAi gene enrichment ranking (RIGER) algorithm, which considers all shRNAs against a specific gene as a “hairpin set”. To identify gene hits that specifically cooperate with MEK inhibitors to reduce viability of KRAS mutant PDAC cells, we used RIGER to compute a depletion score relating to the degree of shRNA dropout between the MEK inhibitor-treated population and the initial untreated population and between the MEK inhibitor-treated population and the vehicle-treated population **(See Figure 1, Supporting Data)**. These depletion scores were combined to determine the top 100 gene hits (shown in red in Figure 1, Supporting Data) that are being utilized to construct the shRNA “mini-pool” for the secondary screen in Aim 2 below.

Reassuringly, the three RAF family genes (shown in blue in Figure 1, Supporting Data) represented three of the top five hits identified in the primary screen. Hyperactivation of MEK by RAF proteins has previously been shown to lead to resistance to MEK inhibitors (Corcoran et al, Science Signaling 2010; Little et al, Science Signaling 2011). The fact that our primary screen identified all three RAF genes as hits is encouraging, in that it suggests that our screen has the ability to identify known genes that cooperate with MEK inhibitors, and thus should have the ability to identify novel gene targets that cooperate with MEK inhibitors in KRAS mutant PDAC.

As our work on the secondary screen (below) proceeds, we are also following up interesting hits from our primary screen that appear to offer promising new mechanistic insights into the molecular susceptibilities of KRAS mutant pancreatic cancer. For example, our finding that all three RAF proteins along with other MAPK pathway components emerged as top hits in our screen have led us to study a key feedback mechanism that leads to MAPK reactivation and resistance to MEK inhibitors in some KRAS mutant cancer cells. Also we found that several members of a key pathway that regulates cellular metabolism and autophagy scored as hits in our primary screen **(Figure 2, Supporting Data)**. As recent data has suggested a critical role for metabolic stress and autophagy-dependence in pancreatic cancer, these data suggest a potentially important therapeutic link between metabolism, autophagy and MEK inhibition that warrants further investigation.

Aim 2/Task 2:

Construction of the inducible shRNA "mini-pool" based on the top 100 gene hits from the primary screen is currently underway. Upon completion, this shRNA mini-pool will be utilized to perform the secondary shRNA-drug screen in an orthotopic mouse pancreatic cancer model as originally proposed. Gene hits from the secondary screen will be validated as originally proposed.

Aim 3/Task 3:

Will be initiated pending completion of Aim 2/Task 2.

KEY RESEARCH ACCOMPLISHMENTS

- Primary shRNA-drug screen completed
- Gene shRNA sets ranked with RIGER algorithm
- Top 100 gene hits identified
- Construction of the inducible shRNA "mini-pool" for secondary screen currently underway.

REPORTABLE OUTCOMES

None at this time. Awaiting completion of project.

CONCLUSION

The primary shRNA-drug screen has been completed and the top 100 genes have been identified. All three RAF genes were identified among the top hits, suggesting that the primary screen has the ability to identify functionally valid hits. Construction of an inducible shRNA "mini-pool" based on the top 100 hits identified in the primary screen is currently underway and will be utilized in the secondary shRNA-drug screen to be performed in an orthotopic model of PDAC.

REFERENCES

1.) Corcoran RB, Cheng KA, Hata A, Faber AC, Ebi H, Coffee EM, Greninger P, Brown RD, Godfrey JT, Cohoon TJ, Song Y, Lifshits E, Hung KE, Shioda T, Dias-Santagata D, Singh A, Settleman J, Benes CH, Mino-Kenudson M, Wong KK, Engelman JA. Synthetic lethal interaction of combined BCL-XL and MEK inhibition promotes tumor regressions in KRAS mutant cancer models. *Cancer Cell*, 23: 121-8 (2013).

2.) Corcoran RB, Dias-Santagata D, Bergethon K, Iafrate AJ, Settlemen J, Engelman JA. BRAF gene amplification can promote acquired resistance to MEK inhibitors in cancer cells harboring the BRAF V600E mutation. *Science Signaling* 3: ra84 (2010).

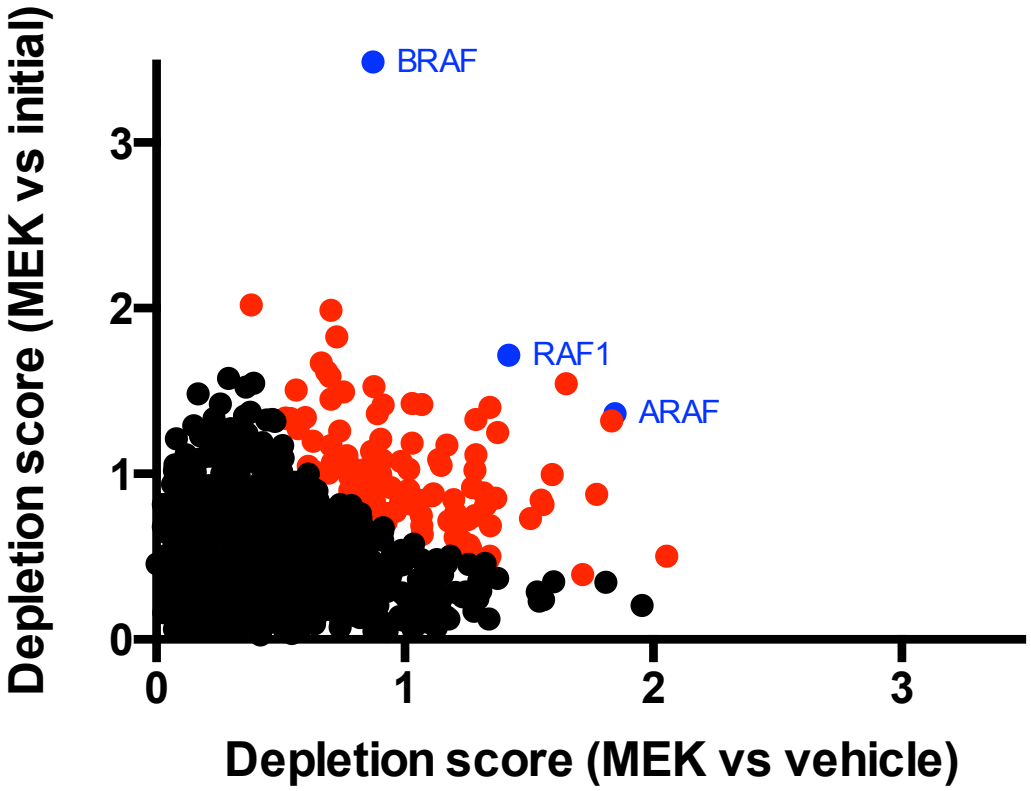
3.) Little AS, Balmanno K, Sale MJ, Newman S, Dry JR, Hampson M, Edwards PA, Smith PD, Cook SJ. Amplification of the driving oncogene, KRAS or BRAF, underpins acquired resistance to MEK inhibitors in colorectal cancer cells. *Science Signaling* 4: ra17 (2011).

APPENDICES

None

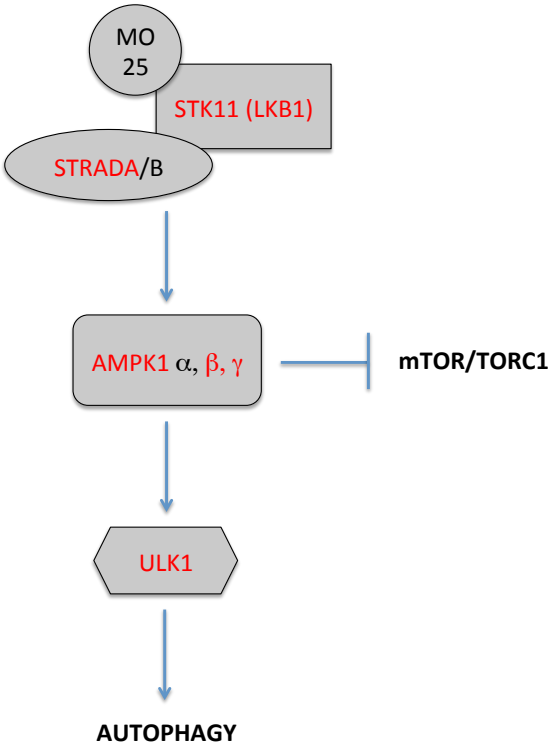
SUPPORTING DATA

Figure 1



Top 100 gene hits as determined in primary shRNA-drug screen. Each gene (consisting of a shRNA "hairpin set") assessed in the primary screen is represented by a circle. The y-axis shows the depletion score for each gene set in the MEK inhibitor-treated population vs. the initial untreated population. The x-axis shows the depletion score for each gene set in the MEK inhibitor-treated population relative to the vehicle-treated population. In red are shown the top 100 gene hits, which are being utilized to construct the shRNA mini-pool for the secondary screen in Aim 2. The three RAF family genes—ARAF, BRAF, and CRAF (RAF1)—which were among the top hits identified in the primary screen, are shown in blue.

Figure 2



The LKB1-AMPK pathway and autophagy. Pathway members that scored as hits in the primary screen are indicated in red.